Non-ohmic Proton Leak Due to Uncoupling Protein Activation by Reactive Oxygen Species

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Abstract: Reactive oxygen species (ROS) are hypothesized to underlie many well-defined diseases and clinically-relevant complications, including those associated with diabetes. Details about ROS production and its specific roles in cellular signaling and tissue damage in living cells in response to nutrients over time are not well understood. Based on the current published data and mathematical models derived from first principles, we present a simple model that captures the responses of mitochondrial respiration, ATP synthesis, and ROS production to glucose stimulation in pancreatic beta-cells. Our model explains experimental observations of the non-ohmic rise in the steady-state proton-leak rate at high membrane potential and its dependence on increased ROS production. We examine the effects that uncoupling proteins have on beta-cell responses to increasing glucose and show evidence that an effective strategy to decrease oxidative stress while increasing insulin secretion may be to increase mitochondrial density while decreasing uncoupling protein activity.

ODEs:

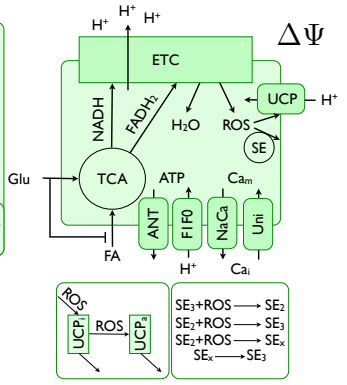
$$\frac{dNADH_m}{dt} = \gamma\left(J_{Glu,N} + J_{FA,N} - J_{O,N} - J_{ROS_P,N}\right)$$
$$\frac{dFADH_{2,m}}{dt} = \gamma\left(J_{Glu,F} + J_{FA,F} - J_{O,F} - J_{ROS_P,F}\right)$$
$$\frac{dADP_m}{dt} = \gamma\left(J_{ANT} - J_{F1F0} - J_{TCA,Glu} - J_{TCA,FA}\right)$$
$$\frac{dCa_m}{dt} = f_m\left(J_{uni} - J_{NaCa}\right)$$
$$\frac{d\Delta\Psi}{dt} = \left(J_{Hres,N} + J_{Hres,F} + J_{Hros,N} + J_{Hros,F} - J_{H,atp} - J_{ANT} - J_{H,leak} - J_{NaCa} - 2J_{uni}\right)/C_m$$
$$\frac{dROS}{dt} = 2\left(J_{ROS_P,N} + J_{ROS_P,F}\right) - J_{SE,3} - 2J_{SE,i} - J_{UCP,a}$$
$$\frac{dSE_3}{dt} = J_{SE,i} + J_{SE,a} - J_{SE,3}$$
$$\frac{dSE_i}{dt} = J_{SE,i} - J_{SE,a}$$
$$\frac{dUCP_a}{dt} = J_{UCP,p} - J_{UCP,a} - J_{UCP,d}$$
$$\frac{dUCP_b}{dt} = J_{UCP,a} - J_{UCP,i}$$

$NAD_{tot} = NAD_m + NADH_m$
$FAD_{tot} = FAD_m + FADH_{2,m}$
$A_{tot} = ADP_m + ATP_m$
$SE_{tot} = SE_3 + SE_2 + SE_x$

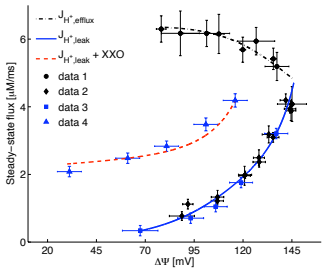
Fluxes:

$$J_{gly} = p_1\sqrt{Glu/1\text{mM}}$$
$$J_{FA} = \frac{p_2}{Glu + p_3}\sqrt{FA/1\text{mM}}$$
$$J_{uni} = p_4 e^{p_5\Delta\Psi}\frac{Ca_i^2}{1\mu\text{M}^2}$$
$$J_{NaCa} = \frac{p_6 Ca_m}{p_7 + Ca_m}e^{p_8\Delta\Psi}$$
$$J_{Glu,N} = p_9 J_{gly}\left(\frac{Ca_m}{p_{10} + Ca_m}\right)$$
$$J_{Glu,F} = p_{11} J_{gly}\left(\frac{Ca_m}{p_{10} + Ca_m}\right)$$
$$J_{FA,N} = p_{12} J_{FA}\left(\frac{Ca_m}{p_{10} + Ca_m}\right)$$
$$J_{FA,F} = p_{13} J_{FA}\left(\frac{Ca_m}{p_{10} + Ca_m}\right)$$
$$J_{O,N} = \frac{p_{14}NADH_m}{p_{15} + NADH_m}\left(\frac{1}{1+\exp\left((\Delta\Psi - p_{16})/p_{17}\right)}\right)$$
$$J_{Hres,N} = \frac{p_{18}NADH_m}{p_{15} + NADH_m}\left(\frac{1}{1+\exp\left((\Delta\Psi - p_{16})/p_{17}\right)}\right)$$
$$J_{O,F} = p_{19}\sqrt{\frac{FADH_{2,m}}{FAD_m}}\left(\frac{1}{1+\exp\left((\Delta\Psi - p_{20})/p_{21}\right)}\right)$$
$$J_{Hres,F} = p_{22}\sqrt{\frac{FADH_{2,m}}{FAD_m}}\left(\frac{1}{1+\exp\left((\Delta\Psi - p_{20})/p_{21}\right)}\right)$$
$$J_{ROS_P,N} = \frac{p_{14}NADH_m}{p_{15} + NADH_m}\left(1 - \frac{1}{1+\exp\left((\Delta\Psi - p_{16})/p_{17}\right)}\right)$$
$$J_{ROS_P,F} = p_{19}\sqrt{\frac{FADH_{2,m}}{FAD_m}}\left(1 - \frac{1}{1+\exp\left((\Delta\Psi - p_{20})/p_{21}\right)}\right)$$
$$J_{Hros,N} = p_{23}\frac{p_{18}NADH_m}{p_{15} + NADH_m}\left(1 - \frac{1}{1+\exp\left((\Delta\Psi - p_{16})/p_{17}\right)}\right)$$
$$J_{Hros,F} = p_{23}p_{22}\sqrt{\frac{FADH_{2,m}}{FAD_m}}\left(1 - \frac{1}{1+\exp\left((\Delta\Psi - p_{20})/p_{21}\right)}\right)$$
$$J_{TCA,Glu} = p_{24} J_{gly}\left(\frac{Ca_m}{p_{10} + Ca_m}\right)$$
$$J_{TCA,FA} = p_{25} J_{FA}\left(\frac{Ca_m}{p_{10} + Ca_m}\right)$$
$$J_{F1F0} = \frac{p_{26}}{p_{27} + ATP_m}\left(\frac{1}{1+\exp\left((p_{28} - \Delta\Psi)/p_{29}\right)}\right)$$
$$J_{H,atp} = \frac{p_{30}}{p_{27} + ATP_m}\left(\frac{1}{1+\exp\left((p_{28} - \Delta\Psi)/p_{29}\right)}\right)$$
$$J_{ANT} = \frac{p_{31}ATP_m}{ATP_m + p_{32}ADP_m}\left(\frac{\Delta\Psi}{\Delta\Psi + p_{33}}\right)$$
$$J_{SE,3} = p_{34}SE_3 ROS$$
$$J_{SE,i} = p_{35}SE_2 ROS$$
$$J_{SE,a} = p_{36}SE_x$$
$$J_{UCP,p} = p_{37} ROS$$
$$J_{UCP,a} = p_{38} UCP_i ROS$$
$$J_{UCP,d} = p_{39}\left(UCP_i - UCP_a\right)$$
$$J_{UCP,a} = p_{40} UCP_a$$
$$J_{H,leak} = p_{41}\left(\Delta\Psi + p_{42}\right) + p_{43} UCP_a$$

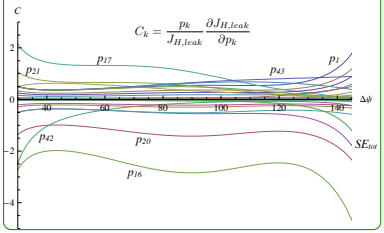
Please also see our other poster, titled **Effects of Free Radicals and Fatty Acids on Pancreatic Beta-Cell Mitochondrial Function**. It is poster number 303.



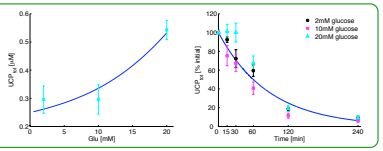
Proton leak rate: A non-ohmic relationship exists between the steady-state proton-leak rate and the membrane potential (blue curve). Exogenous ROS production (+XXO) increases this proton conductance at each membrane potential (red curve, calculated by adding $J_{ROS,ex}$ = 0.4μM/ms to the ROS production rate). Data from Affourtit, C and MD Brand, *Biochem. J.* **393**:151-159, 2006 (data 1 & 2) and Echtay, KS *et al.*, *Nature*, **415**:96-99, 2002 (data 3 & 4) are shown for comparison.



Here we show the control coefficients (C_k) for each parameter (p_k) related to the $J_{H,leak}$ curve.



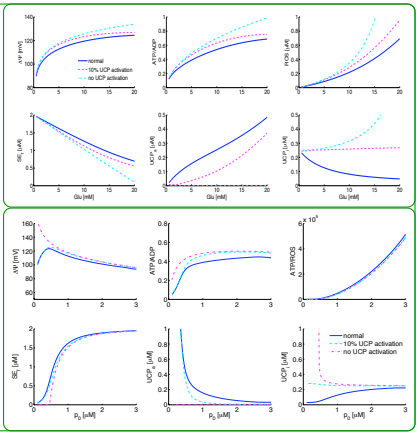
Uncoupling proteins: As glucose increases, ROS signals for increased UCP production, and total UCP content increases. Halting protein synthesis gives the UCP decay curve with a half-life of approximately 60 minutes. Data from Azzu, V *et al.*, *Biochim. Biophys. Acta*, **1777**:1378-1383, 2008 is shown for comparison.



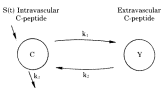
UCP activation and mitochondrial density: The ATP/ADP ratio and ROS levels increase in response to increasing glucose. These responses are signals for insulin secretion. To improve insulin secretion in the short term, UCP activation could be inhibited (top). This would prevent energy diversion from ATP production, but it would also increase ROS production, which could cause greater oxidative damage in the long term.

Increasing mitochondrial biogenesis increases the ATP/ROS ratio (bottom). This occurs because the greater number of mitochondria share the nutrient load, and are able to produce ATP in a less stressful way.

Therefore, increasing mitochondrial density (p_0) while decreasing uncoupling protein activity may be an effective strategy to decrease oxidative stress while increasing insulin secretion.

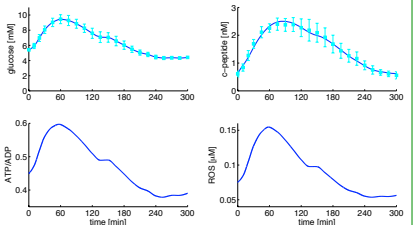


C-peptide secretion model: Our model can be incorporated into a c-peptide model, originally proposed by Eaton, RP *et al.*, *J. Clin. Endocrin. Metab.* **51**:520-528, 1980, to predict the insulin secretion rate and provide a quantitative description of β-cell function for a single individual. To illustrate, we show an example where we used the model parameters (k_1, k_2, and k_3) estimated by Van Cauter, E *et al.*, *Diabetes*, **41**:368-377, 1992, a glucose profile measured by Breda, E *et al.*, *Diabetes*, **50**:150-158, 2001, and the corresponding ATP/ADP and ROS profiles predicted by our model, in an optimization problem to determine simple secretion rate parameters and fit the c-peptide profile.



$$\frac{dC(t)}{dt} = -(k_1 + k_3)C(t) + k_2 Y(t) + S(t)$$
$$\frac{dY(t)}{dt} = k_1 C(t) - k_2 Y(t)$$
$$S(t) = -k_1 C(t_0)e^{-k_2(t-t_0)} - k_1 k_2 \int_{t_0}^{t} C(s)e^{-k_2(t-s)}ds + \frac{dC}{dt} + (k_1 + k_3)C(t)$$

$$S(t) = k_a\frac{ATP}{ADP} + k_r ROS - k_b$$



Conclusions: The model we developed goes beyond the models upon which it was based by incorporating ROS production and the activity of scavenging enzymes and uncoupling proteins. Its simplicity is an advantage in that it allows easy manipulation and transference, making it useful in pursuing research investigating the integrative physiology of mitochondria in various tissues. Our model is consistent with a number of experimental observations reported in the literature: the most notable of which is the non-ohmic proton-leak rate as a function of mitochondrial membrane potential. Having been developed for pancreatic β-cell mitochondria, the model allowed us to make inferences and propose hypotheses related to insulin secretion and the effects of ROS, uncoupling proteins, and mitochondrial density. Our results suggest that increasing mitochondrial density while decreasing uncoupling protein activity may be an effective way to increase glucose-stimulated insulin secretion. Furthermore, our model can be applied to predict the c-peptide and insulin secretion rate in a single individual based on a time-course profile of blood-glucose concentrations. This application could be useful in a clinical setting to quantify β-cell function.

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